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SENSORY STRUCTURES OF THE ANTENNAE OF *NANNOTRIGONA TESTACEICORNIS* (APIDAE: MELIPONINAE)[1]

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Abstract.—The total number and distribution per antennal flagellomere of sensilla placodea (olfactory disks), sensilla coeloconica, sensilla ampullacea and sensilla campaniformia were determined in workers of *Nannotrigona testaceicornis* Lepeletier a stingless bee species quite common in Brazil. The distribution of the sensilla was uniform, with the largest number occurring in flagellomere 10 and gradually decreasing in the direction of the basal flagellomeres in a way similar to that observed in *Scaptotrigona postica* Latreille.

Nannotrigona testaceicornis had a larger number of sensilla ampullacea and a smaller number of sensilla coeloconica and sensilla campaniformia than *Scaptotrigona postica*. Although *Nannotrigona testaceicornis* does not communicate through the formation of pheromone trails, this species presents a larger quantity of sensilla placodea (relative to the length of the flagellum) than *Scaptotrigona postica*.

Nannotrigona testaceicornis, a stingless bee species quite common in Brazil, is found from the north of Paraná up to Mexico (Nogueira Neto, 1970). This species presents on average populous colonies, generally consisting of 2,000 to 3,000 individuals (Lindauer and Kerr, 1960).

Nannotrigona testaceicornis has been studied from different aspects such as oviposition behavior (Sakagami and Zucchi, 1966), communication (Kerr and Esch, 1965), glandular system (Cruz-Landim, 1967), taxonomy (Moure, 1951), nest structure (Nogueira Neto, 1970) and morphometry (Cunha, 1973). On the other hand, only little information exists with respect to the antennal sensilla placodea of the workers of this species (Johnson and Howard, 1987).

In order to expand the knowledge about the outer morphology of Brazilian stingless bees, we determined the number and distribution of sensilla placodea, sensilla coeloconica, sensilla ampullacea and sensilla campaniformia of the antennae of *Nannotrigona testaceicornis* workers.

MATERIAL AND METHODS

Samples of *Nannotrigona testaceicornis* Lepeletier workers were collected from colonies maintained at the Animal House of the Biosciences Institute of Rio Claro. The bees were anesthetized and killed in an ether chamber, fixed in modified Karnovsky (2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2) for 24 hours and stored in 70% alcohol. The antennae were separated from

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the head, treated with ultrasound for 1 minute to remove dirt particles and glued to a metal support.

The antennae were then sputtered with a thin gold layer using an Edwards Sputter apparatus, model S_{150B}, and observed with a model T_{330A} Jeol scanning electron microscope of the Chemistry Institute, UNESP, Araraquara.

For sensilla placodea (olfactory disks) counts, the antennal flagellomeres were separated and opened with the aid of two entomology pins and mounted on balsam between a slide and a coverslip. Each flagellomere was photographed with a Zeiss photomicroscope II, and the film was projected onto a paper screen where the sensory structures were counted.

The ratio number of sensilla placodea to length of the flagellum was also studied. The length of the flagellum was measured under a dissecting microscope equipped with an optical micrometer.

Ten worker bees were used for the observations and counts.

RESULTS

In flagellomere 10 the set of sensilla campaniformia was located above the sets of sensilla coeloconica and sensilla ampullacea (Fig. 1A), with these two types of sensilla (coeloconica and ampullacea) being clearly distinguishable.

The total number of sensilla ampullacea in *Nannotrigona testaceicornis* workers was larger than that of sensilla coeloconica, with the largest number of these sensilla and of sensilla placodea occurring in flagellomere 10 and decreasing in the direction of the most basal flagellomeres (Table 1).

The set of sensilla campaniformia was only observed in flagellomere 10, having on average 5.800 ± 0.421 units (Table 1). No set of these sensilla was observed in the other flagellomeres below flagellomere 10 of the antennae of *Nannotrigona testaceicornis* workers (Fig. 1B), and an isolated sensilla campaniformia rarely occurred.

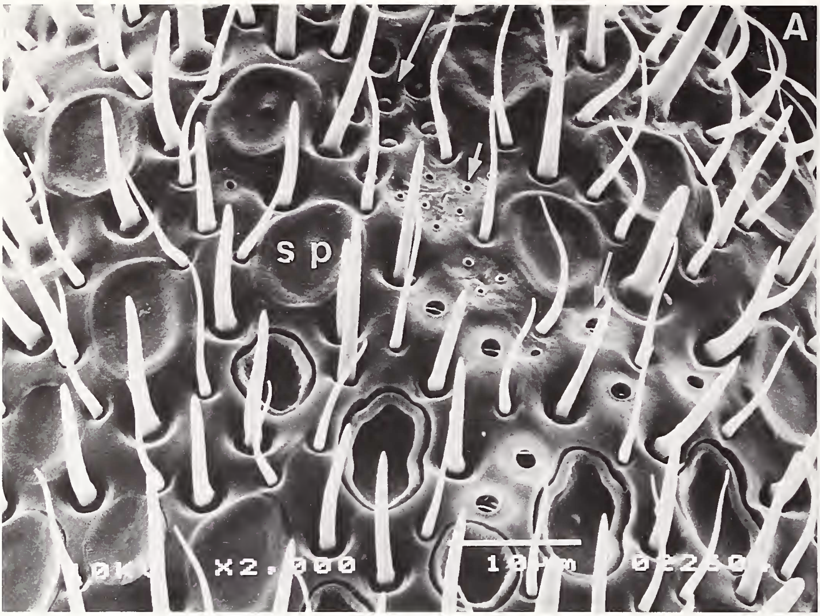
DISCUSSION

The location of the set of sensilla campaniformia above the sets of sensilla coeloconica and sensilla ampullacea in flagellomere 10 of the antennae of *Nannotrigona testaceicornis* workers is similar to that observed in *Scaptotrigona postica* (Stort and Barelli, 1981; Stort and Moraes-Alves, 1997) and differs from what occurs in *Apis mellifera* where the set of sensilla campaniformia is located laterally to the set of sensilla coeloconica + ampullacea (Dietz and Humphreys, 1971; Stort and Moraes-Alves, 1997).

The morphological differences between sensilla coeloconica and sensilla ampullacea observed in *Nannotrigona testaceicornis* are similar to those observed in *Scaptotrigona postica* (Stort and Barelli, 1981).

Nannotrigona testaceicornis workers possess a smaller total number of sensilla coeloconica and a larger total number of antennal sensilla ampullacea (Table 1) than *Scaptotrigona postica* (Stort and Moraes-Alves, 1997).

Regarding the total sum of these two types of sensilla (sensilla coeloconica + sensilla ampullacea), *Nannotrigona testaceicornis* presents a smaller quantity (mean: 48.100 ± 5.606) than *Scaptotrigona postica* (mean: 52.800 ± 5.266). According to



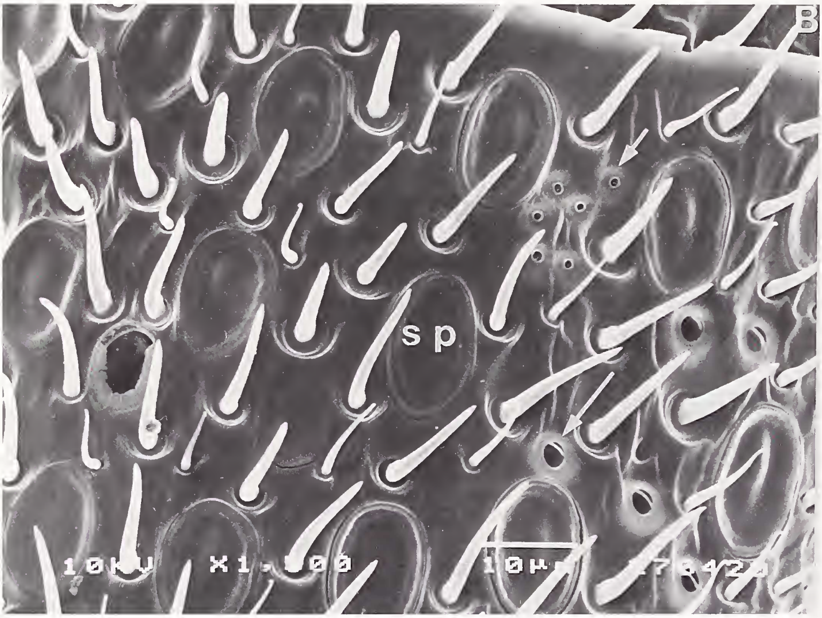


Fig. 1. Scanning electron microscopy micrographies of the antennal flagellomeres of *Nannotrigona testaceicornis* worker. A—flagellomere 10 showing sensilla campaniformia (long arrow), sensilla ampullacea (short arrow), sensilla coeloconica (median arrow) and sensilla placodea (S P). B—flagellomere 9 showing sensilla ampullacea (short arrow), sensilla coeloconica (long arrow) and sensilla placodea (S P).

Table 1. Means (±standard deviations) of the number of sensilla placodea, sensilla coeloconica, sensilla ampullacea and sensilla campaniformia of the antennal flagellomeres (F) of *Nannotrigona testaceicornis* workers.

	Sensilla placodea	Sensilla coeloconica	Sensilla ampullacea	Sensilla campaniformia
F_{10}	145.600 ± 5.274	4.300 ± 0.823	6.800 ± 0.918	5.800 ± 0.421
F_9	104.800 ± 4.638	3.900 ± 0.994	5.400 ± 1.577	—
F_8	103.600 ± 4.247	3.500 ± 0.707	4.300 ± 2.057	—
F_7	87.300 ± 4.762	3.400 ± 0.843	3.100 ± 0.994	—
F_6	87.500 ± 3.719	2.300 ± 1.159	3.200 ± 1.988	—
F_5	76.800 ± 7.656	1.800 ± 0.788	1.900 ± 0.737	—
F_4	76.700 ± 4.990	1.700 ± 0.483	1.800 ± 1.135	—
F_3	64.300 ± 4.243	0.400 ± 0.699	0.300 ± 0.483	—
F_2	48.200 ± 4.825	—	—	—
Total	794.800 ± 22.592	21.100 ± 2.960	27.000 ± 4.876	5.800 ± 0.421

Kuwabara and Takeda (1956), these structures are hygroreceptor organs, a fact that was confirmed by Lacher (1964). *Nannotrigona testaceicornis* would therefore be less sensitive in detecting the degree of environmental humidity than *Scaptotrigona postica.* In contrast, Africanized *Apis mellifera,* which present on average 71.900 ± 7.311 sensilla coeloconica + ampullacea (Stort and Rebustini, 1997), appear to be more sensitive in detecting humidity than these two stingless bee species.

Both *Nannotrigona testaceicornis* and *Scaptotrigona postica* have one set of sensilla campaniformia in flagellomere 10 and rarely present other sensilla of this type, even isolated, together with sensilla coeloconica and sensilla ampullacea in the flagellomeres below number 10.

Nannotrigona presents a smaller quantity of sensilla campaniformia (Table 1) than *Scaptotrigona* (Stort and Moraes-Alves, 1997). Therefore, this species should be less sensitive to temperature and CO_2 since these sensilla are related to the perception of these environmental parameters (Dietz and Humphreys, 1971). According to Stort and Rebustini (1997), Africanized *Apis mellifera* workers have a larger quantity of sensilla campaniformia (mean: 18.700 ± 2.710) since these sensilla are present in all antennal flagellomeres, and this species may therefore be more sensitive to these environmental factors than the two stingless bee species mentioned here.

The total number of sensilla placodea (olfactory disks) per antenna in *Nannotrigona testaceicornis* was on average 794.800 ± 22.592 (Table 1), a number 30.397% smaller than that observed in the antenna of *Scaptotrigona postica* (Stort and Barelli, 1981) and 327.126% smaller than that observed in Africanized *Apis mellifera* (Stort, 1979). The total number of sensilla placodea of *Nannotrigona testaceicornis* obtained in the present study disagrees completely with the number (1,858 ± 338) found by Johnson and Howard (1987).

The total number of sensilla placodea, however, when considered in relation to the length of the antenna which was determined by calculating the number of sensilla placodea/flagellum length ratio, showed a difference. The ratios obtained were 24.380 for *Nannotrigona,* 24.102 for *Scaptotrigona* and 39.877 for Africanized *Apis.* This means that *Nannotrigona* presents, proportionally to the size of the flagellum, a slightly larger quantity of antennal sensilla placodea than *Scaptotrigona,* and *Apis* presents a larger quantity than these two. It is important to analyze the number of sensilla in relation to the size of the bee since Johnson and Howard (1987) have shown that these two characters are highly correlated.

According to Kerr (1969), *Nannotrigona testaceicornis* has a barely evolved system of communication between workers. It has been shown that the worker that has found a food source enters the colony and produces a characteristic sound. The workers surrounding this bee immediately start to produce the same sound and in less than one minute the entire colony will be buzzing, imitating this sound, and at this time several bees leave the colony looking for food (Kerr and Esch, 1965). Thus, the bee that found the source alerts the colony in terms of the existence of this source but does not provide any indication of the location. The source is only found based on the smell of the food.

In contrast, *Scaptotrigona postica* presents a more evolved communication system. The bee that found the food source leaves a scent trail between the source and the colony, marking flowers, leaves and branches every 1 to 2 meters with a drop of secretion (rich in compounds such as 2-heptanone, 2-nonanone and benzaldehyde) produced by the mandibular glands (Lindauer and Kerr, 1958; Kerr et al., 1963). Due to the type of communication, i.e., formation of a pheromone trail, it may be expected that *Scaptotrigona postica* presents a larger quantity of antennal sensilla placodea (olfactory disks) than *Nannotrigona testaceicornis,* which is not the case when the size of the flagellum is also considered. In this respect, the number of antennal sensilla placodea of *Scaptotrigona postica,* in addition to not agreeing with the type of communication they possess when compared to *Apis mellifera* and *Melipona quadrifasciata* (Silva de Moraes and Cruz-Landim, 1972; Stort and Barelli, 1981), also does not agree with the type of communication they possess when compared to *Nannotrigona testaceicornis.* These results support the observation that the number of antennal sensilla placodea is not simply related to the use of pheromones for the recruitment of individuals to the food source, but may play a broader role in the complex number of activities performed by bees (Johnson and Howard, 1987).

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